[0200] Different chromatographic separations can be accomplished by stacking multiple blocks having different separation characteristics from one another. For example, the study of a proteome may involve the analysis of complex mixtures of up to several thousand different proteins within a sample. Analysis of these complex mixtures requires a multi-dimensional separation of the components of the mixture in order to identify and quantify the levels of the specific proteins. 2-dimensional ("2D") liquid chromatography ("LC") separations can be accomplished using multiple stacked separation blocks wherein the first block contains an LC separation phase based on ion exchange (strong cation exchange) or size exclusion separation modes. The second separation block contains a separation phase based on a reversed phase separation mode. In the case of ion exchange, a complex protein sample may be separated using an increasing salt concentration in the elution buffer over time. By performing a salt gradient in a stepwise method, for example, fractionation of complex mixtures from the first phase being an ion exchange phase to a second phase being a reversed phase provides for a 2D separation of the sample. Further combining this separation with mass spectrometry/mass spectrometry provides for 2 additional dimensions of separation based on mass/charge ratio.

[0201] **FIG. 20A** shows two blocks stacked one on top of the other. A sample is delivered to a plurality of separation channels **8** in a first separation block **1** at the entrance orifices **5**. The sample may be delivered via a delivery device such as a conduit or from an exit orifice of an upstream separation block, or the like. The channel **8** is filled with a polymer monolith **3** for chromatographic separation of the sample. The sample travels through the separation channels **8** of the separation block **1** and is delivered at the interface **7** to the corresponding entrance orifices **5** of the channels **8** of a second separation block **2** which can be filled with a polymer monolith **4** having the same or different separation characteristics as that of the first separation block **1**. The sample is eluted through exit orifices **6** to a fluid receiving device. The fluid receiving device can be a fluid control device, an electrospray device, a detection device, or the like. The fluid control device can be used to adjust the pressure of the sample stream by, for example, applying a positive pressure at one end or a negative pressure at the other end. For example, in a proteomics sample array a first separation device having an ion exchange polymer monolith can be stacked on top of a second separation device having an organic solvent separation polymer monolith coupled to a mass spectrometer.

[0202] The separation characteristics of the polymer separation block of the present invention can be enhanced by coupling multiple blocks, such as in a stacked arrangement, together upstream of an electrospray chip. Several structural features of the blocks enhance the ability to couple multiple blocks together and couple to an electrospray chip. As can be seen in **FIG. 20A**, the entrance orifice **5** of the separation block **2** can be designed in the shape of a well such that it is compatible with the exit orifice **6** of the corresponding separation block **1** or, the exit orifice **6** of the separation block **12** can be mated with the entrance orifice **10** of an electrospray device **11**, as shown in **FIG. 20B**. These features facilitate easy alignment and coupling of one block to another block and/or to a through-substrate electrospray device. They are also useful for the alignment and supporting of a gasket material **9**, as shown in **FIG. 20A**, between the blocks or between the block and the electrospray device (not shown). The gasket can aid in preventing cross-contamination and leakage from one separation channel to another separation channel of adjacent blocks or adjacent electrospray chip device.

[0203] Accordingly, the sample can be transferred from one block to another to effect chromatographic fractionation from one block to one or more blocks and can also be coupled to the electrospray device of the present invention for further separation and sample preparation and/or to a detection device for spectroscopic detection such as, UV absorbance, laser induced fluorescence, and evaporative light scattering.

[0204] The polymer monolith can be prepared in accordance with the procedures noted above with respect to the in situ preparation of a porous polymer monolith within the through-substrate channels and/or reservoir of a microchip electrospray device of the present invention. In a preferred formulation of the present invention the polymer monolith of the separation block is formed from one or two monovinyl monomers with the addition of a cross-linker. Preferred monovinyl monomers include styrene, vinylbenzyl chloride, vinylacetate, alkyl methacrylates and glycidyl methacrylates. Preferred cross-linkers include divinyl monomers, such as divinylbenzene, ethyleleglycol and dimethacrylete. The preferred formulation has a 20-50 volume/volume percent cross-linker in the monomer mixture.

[0205] The preferred initiators in an 8-24 hour reaction under heating with a purge of an inert gas include 2'2-azobisisobutyronitrile (0.2-0.5 w/v %: 45-75° C.); and benzoyl peroxide (0.2-0.5% w/v %; 60-80° C.). For photo polymerization the above initiators can also be used as photo sensitized initiators at UV wavelengths of 2000-4000 Å.

[0206] The preferred porogen is a mixture of a relatively less polar organic solvent, for example an alcohol, such as 1-propanol and a more polar organic solvent, such as formamide. Preferably, the monomer to porogen ratio is about 40 to about 60 v/v.

[0207] The finished monolith pore size is preferably from 1 to 3 microns and the monolith has a porosity from 45 to 65 v/v %. The separation channel is typically less than ten centimeters, preferably less than five centimeters, more preferably less than three centimeters, more preferably less than one centimeter and most preferably less than five millimeters. Separation channels having an inner diameter of less than one millimeter are preferred, more preferably less than 0.5 millimeters, more preferably less than 0.3 millimeters, more preferably less than 0.2 millimeters, and most preferably less than 0.1 millimeter.

[0208] The chromatographic resolution of the polymer monolith is significantly independent of the flow rate of the mobile phase. The quality of the separation does not change when increasing the flow rate so long as the same gradient volume is maintained. For example, E. C. Peters et al. in Rigid Macroporous Polymer Monoliths, Advanced Materials, 1999, 11, No. 14, which is herein incorporated by reference in its entirety, disclose the separation of three proteins on a poly (styrene-co-divinylenzene) monolithic column at flow rates of 5 and 25 mL/min. using a column having a 50×8 mm inner diameter where the mobile phase is a linear gradient from 20 to 60 percent acetonitrile in